THE PYRENOID OF ANTHOCEROS

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The single chloroplast of the cells of the gametophyte of the Anthocerotes, with its pyrenoid-like central region, has long been known. Notwithstanding this, detailed descriptions of these chloroplasts and pyrenoids and comparisons with those of the algae, on the one hand, and with the chloroplasts of the liverworts on the other, seem to be lacking.

As early as 1851 von Mohl (14) called attention to the fact that there were "wohl 50 bis 100 Amylumkörner" in the chloroplasts of Anthoceros, but he did not relate them to the starch aggregations to be found in the green algae.

The voluminous earlier literature on the morphology of Anthoceros, as far as I can determine, contains only bare mentions of the chloroplasts and pyrenoids. Leitgeb (9) in fact seems to have made no reference whatever to these structures.

Schimper (18) states that the pyrenoids of Anthoceros "zeigten nach Entfernung der Stärke durch Verdunklung nur noch corrodierte, unregelmässig eckige Umrisse." His figures 15 and 16 are apparently surface views of entire, living cells and show only diffuse central regions which have no resemblance to the plastids as seen in stained material.

Davis (5) in an account of the nuclear division and the fission of the chloroplasts in the spore mother cells of *Anthoceros laevis* makes no reference to a pyrenoid and nothing in his figures suggests such a structure. He is unable to identify plastids in the archesporial cells and only as these cells become spore mother cells can the single, very minute chloroplast be identified. This chloroplast enlarges rapidly and undergoes two fissions, thus forming the four chloroplasts of the spore tetrad. The large chloroplasts of the mature spores are filled with conspicuous starch grains, becoming thus "storage vesicles of starch."

Campbell (3, 4) has made the most important recent contribution

to our knowledge of the chloroplasts of the Anthocerotes. He has shown that in certain tropical Anthoceros forms more than one chloroplast is usually found in the cells of the gametophyte and that in those cells showing the greatest increase in the number of the chloroplasts the pyrenoids are poorly defined or lacking. Referring to a species collected in Buitenzorg in which all of the interior cells of the thallus showed multiple chloroplasts, not infrequently as many as eight in a cell, he says: "The pyrenoid, usually so conspicuous in the chromatophores of the Anthocerotes, seems to be quite absent and in this respect, as well as the increased number of the chromatophores there is a close approach to the chromatophores of the other archegoniates." He finds that in a Javanese form, which he calls *Megaceros Tjibodensis*, as many as twelve chloroplasts are sometimes present in a single cell. In most of the cells, however, two to four were to be seen and occasionally but one. No trace of a pyrenoid could be recognized. In *Megaceros Salakensis* the chloroplasts of the upper surface of the thallus are larger and have aggregations of starch grains suggesting pyrenoids though less definite than in Anthoceros. The interior cells usually have four to six choloroplasts which lack pyrenoids. The author intimates that small starch grains are present in these chloroplasts (". . . nor were large starch granules observed") though he does not make a direct statement to that effect.

Sapĕhin (16, 17) has shown that the presence of a single chloroplast in the sporogenous cells of Anthoceros is not peculiar to this group. He reports that the sporogenous tissues of Selaginella, Isoetes, and the mosses as well as the meristematic cells of certain Bryophytes have but a single chloroplast.

Schmitz (19) gave the name "pyrenoid" to the kernel-like bodies in the algal chloroplast which have a different texture and staining reaction than the surrounding plastid cytoplasm. Pyrenoids are found in the diatoms, certain of the Rhodophyceae, the Euglenidae, and the green algae.

De Bary as far back as 1858 (1) studied the pyrenoid in Spirogyra and determined by means of the iodin reaction that it was surrounded by an outer layer of starch, and by means of sugar and sulphuric acid claimed to show that the central part was protein.

Schmitz (19) described the pyrenoid as a comparatively dense, homogeneous mass which is differentiated from the chlorophyll-bearing protoplasm. Its microchemical reactions led him to conclude

that it is similar chemically to nuclein. He describes the fission of the chloroplast and pyrenoid in Hyalotheca and believes that fission is the common mode of multiplication of pyrenoids although he is also of the opinion that they may arise from the cytoplasm *de novo*. Although recognizing the close relation of the pyrenoid to starch formation he insists that it takes no direct, morphological part in the process. He believes the starch to be deposited at all times in the clear zone immediately surrounding the pyrenoid.

The angular shape of the pyrenoid of *Bryopsis plumosa* as well as of other forms, together with the difficulty of proving its fission, led Schimper (18) to the belief that the pyrenoid is of the nature of a protein crystal. He agrees essentially with Schmitz as to the mode of the formation of starch about the pyrenoid but disagrees in part as to the mode of multiplication, believing that new pyrenoids are formed only as are new crystals, *de novo*.

Boubier (2) has proposed that not only the compact central body but also the clear area about it should be regarded as the pyrenoid. The clear area he believes to be penetrated by radiating, granular strands. Of these granular strands he says: "J'assimile cette substance granuleuse a un leucite dans les mailles duquel se depose l'amidon."

Wiesner (22) had earlier regarded the interior of the pyrenoid as made up of plastids of the nature of leucoplasts, each plastid giving rise to a starch grain.

Timberlake (20) is "inclined to the view that the pyrenoid is a active body, differentiated from the chlorophyll-bearing cytoplasm, which in co-operation with the latter acts as the basis for starch formation." His work on Hydrodictyon showed that segments split off from the pyrenoid in concentric scale-like discs. By the deposition of starch within these segments starch grains are formed which are considerably larger than the pyrenoid segment. During this process of starch formation the pyrenoid undergoes continual growth but at the same time is being reduced in size by the cutting off of rudimentary starch grains. The pyrenoid stains red with the safranin of the triple stain but at the time of the cutting off of the segments, the material cut off takes the violet stain, indicating thus its starchy nature. In Rhizoclonium, Cladophora, and Oedogonium he finds (21) that the pyrenoid often becomes split into halves and that either or both of these halves may become a starch grain, or, in some instances, the

entire pyrenoid may become a single starch mass. In both of these latter cases the entire pyrenoid becomes starch.

Lutman (11) has been unable to prove with certainty the cleaving off of such rudiments of starch grains in Closterium. He finds a cleavage of the pyrenoid but is not positive that the resulting segments give rise to starch grains. He seems inclined to the view that they may give rise to new pyrenoids. His results make it clear that the pyrenoid in Closterium is not homogeneous but nearly always shows areas which absorb the stain in a varying degree. Often faintly stained lines in the pyrenoid are continuous with the clefts between the surrounding starch grains suggesting a relation between these lines in the pyrenoid and the cleavage of segments. In other cases the entire pyrenoid may break up into "lens-shaped segments."

Yamanouchi (23) has described what he regards as a new species of Hydrodictyon in the cells of which are to be seen numerous ovoid or spheroid chloroplasts. These chloroplasts "have two functions, one to produce characteristic pyrenoids and the other to form reserve starch grains." Starch formation, according to Yamanouchi, here has no relation to the pyrenoid but occurs at or near one side of the small chloroplasts, in much the same manner as the starch grains are formed by the leucoplast of Phajus. If these results are substantiated by further study we shall have the most curious phenomenon of a pyrenoid in the green algae having no relation to starch formation and the starch formed by minute chloroplasts in a manner apparently the same as in the chloroplasts and leucoplasts in the higher plants. Such evidence would seem at least sufficient to exclude this alga from the genus Hydrodictyon.

I have shown (12) that the pyrenoids of Tetraspora may split up to form several small starch grains, or the entire pyrenoid may become converted into a single starch mass much in the same manner as Timberlake (21) has described for Rhizoclonium and certain other algae.

Due to the recent researches of Lewitsky (10), Guilliermond (8), and others, a voluminous literature on the subject of chondriosomes in plant cells has been developed. The last mentioned authors claim to have established the origin of plastids from chondriosomes. It is, however, beyond the province of this paper to deal with the origin of plastids as such.

Description of Observations

The material for this study was collected in the vicinity of Ithaca, N. Y., in August, 1912. The fixation was carried on with the aid of an air pump, thus insuring quick penetration of the fixative into the intercellular cavities of the thallus. The killing agents used were Flemming's strong osmic acid mixture, medium strength osmic acid mixture as used by Strasburger, and Merkel's killing solution.

For the study of starch formation Merkel's solution gave rather the best results. The hydrogen peroxide bleaching mixture apparently exerts a weak hydrolytic action for a few days' exposure of preparations of Anthoceros, Conocephalum, Reboulia, and other liverworts containing starch to the ordinary bleaching solution (hydrogen peroxide one part and 50 per cent alcohol one part) is sufficient to remove all of the starch, or at least to render it incapable of staining either with iodin or with Flemming's triple stain. In order therefore to keep the starch as nearly as possible as it is in the living cell it is desirable to avoid the use of killing solutions which necessitate a bleaching of the fixed material. Merkel's solution answers this requirement, and other than a slight difficulty in staining achromatic figures following its use, this killing agent compares very well indeed with the osmic acid mixtures.

The chloroplast of the gametophyte of *Anthoceros laevis* is, in general, lens- or disc-shaped (figures 1 and 4). The thickness of the disc varies greatly, ranging from more than half its diameter to less than one eighth (figures 2 and 3). As will be suspected, the thicker chloroplasts are associated with abundant starch formation and are to be found in the mature areas of the thallus and close to the surface, while the flatter plastids seem to be located in cells which are more or less dormant and in the interior cells of the thick, mature thallus. Usually the surface of the disc next to the cell wall is convex while there is a tendency toward concavity on the side facing the vacuole, though a noticeable bulge is nearly always present opposite the pyrenoid.

The chloroplast varies in size according to the size of the cell in which it is situated. In the region of the growing point where the cells are small, the plastids measure about 10 microns in diameter by 4 in thickness (figure 6). In the large cells of the fully grown areas of the thallus they may be as large as 40 by 15 microns (figure 8).

Near the central part of the chloroplast, though somewhat nearer the vacuole than the cell wall, is located a more or less compact group

of minute, flattened spindle- or scale-like bodies forming the so-called "pyrenoid" (figures 1, 2, and 3). An examination of a great variety of cells in various stages of activity shows definitely that there is never a homogeneous unsegmented center about which starch grains are formed as is the case with the pyrenoids of the green algae. Wherever a pyrenoid is to be seen it always appears as a group of from 25 to 300 very minute bodies rather than a single, large, spherical body. This group of bodies is often so compact as to appear practically homogeneous when viewed with a four millimeter objective although frequently, when they are not crowded, the bodies can be seen with a sixteen millimeter objective. It seems nevertheless possible that with poor fixation and staining the individual bodies might at times be unrecognizable even with an oil immersion objective.

These pyrenoid bodies, as I shall call them in the following pages, stain red or reddish with Flemming's triple stain, the brilliancy of the stain depending apparently upon the activity of the cell and plastid. In cells which are semi-dormant where growth is very slow and the pyrenoid bodies are closely crowded, they stain a dull reddish color. When loosely aggregated, as they are usually when photosynthesis is very active, they appear as flattened, scale-like bodies which stain a brilliant red. Starch grains which nearly always surround them under these conditions always take the violet stain. With Millon's reagent the central aggregation of bodies stains orange and with nitric acid the characteristic xanthoproteic reaction is obtained,—the surrounding starch grains in both cases remaining colorless. These reactions indicate as definitely as our present microchemical tests permit the protein nature of the pyrenoid bodies.

As has been suggested above, considerable difference exists in the degree of aggregation of the bodies which make up the pyrenoid. Those in the cells which contain little or no starch, or in other words, in which photosynthesis is not active, show always a compact mass of pyrenoid bodies. Figure 6 shows such an aggregation in a small cell near the growing point. The deeply lying cells of the mature thallus usually have such dense aggregations (figures 1 and 19). It is of interest in this connection to note that the peripheral cells, even on the lower surface of the thallus, always show much more active photosynthesis than those submerged in the thallus, a condition which is without doubt due to insufficient aeration of the interior regions.

In certain thalli, which are apparently in a dormant condition,

practically all of the cells have these densely aggregated pyrenoids. In such cases the plastids are very thin and the pyrenoid bodies closely aggregated (figure 3).

The pyrenoid bodies in these dense aggregates are usually less angular than those in the looser, active pyrenoids. The bodies, though still of the shape of a flattened disc, have rounded edges (figures 1 and 19). Those in the active pyrenoids are loosely aggregated and are flatter, with sharp, angular edges. So loosely are they aggregated in some plastids that they can only be distinguished from the inner starch grains by their staining reaction and possibly by their smaller size (figure 8).

This difference in the shapes of the active and quiescent pyrenoid bodies shows very clearly that the shape of the bodies is not due to the mutual pressure of plastic structures in the compact aggregates, for if this were the case we should of course find the greatest angularity in the compact masses.

Due to their minuteness I have been unable to find conclusive evidence of the formation of new pyrenoid bodies by the fragmentation or fission of preexisting bodies. They however often lie very closely together and overlap in such a manner as to suggest very strongly such an origin (figure 7). Indeed, the form and the arrangement of the pyrenoid bodies and the starch grains as a whole suggests very strikingly an origin by fission. Their form is very commonly that of an elongated disc or spindle, convex on the outside and concave on the inside. The general form of the mature starch grains as well as that of the loosely aggregated pyrenoid bodies is the same as that of compact masses frequently seen among active plastids (figure 7). These compact pyrenoids among looser, active ones have apparently been slower in arriving at the proper conditions to deposit starch. Their pyrenoid bodies are brilliant red and very similar to those in active pyrenoids with abundant surrounding starch. It is difficult to conceive that bodies overlapping one another in such definite order as do the pyrenoid bodies and starch grains could have arisen *de novo* in the cytoplasm of the plastid. Nevertheless the fact of their being invisible in the very young assimilative cells in the area of intercalary growth of the sporophyte would seem to point to the conclusion that there, at least, the pyrenoid bodies are lacking entirely and later arise *de novo*.

The direct transformation of pyrenoid bodies into starch grains is easily followed. In many plastids there is a gradual transition of the

color reaction, from the brilliant red of the pyrenoid bodies to the blue of the starch grains. There is no change in the shape of the bodies during this change in color though there may be a very slight increase in the size of the grains during the transition (figure 8) which is of course to be expected. There can be no doubt that the red-staining pyrenoid bodies become transformed directly into starch grains without any change in their form. After this change in the chemical composition of the bodies there is a gradual increase in the size of the starch grain until the mature grain results. During this increase in size the grain has moved outward and when it has reached the periphery its growth ceases and it slowly disappears.

The chloroplasts of the sporophyte, in the vegetative cells not shielded by the sheath of the gametophyte, do not differ in structure from those of the gametophyte, though they average considerably smaller in size. The smaller chloroplasts of the gametophyte could not be distinguished in any way from the larger sporophytic chloroplasts (figures 6 and 17).

No pyrenoids however are recognizable in the plastids of the cells of the foot, nor in the embryonic assimilative tissue of the sporophyte immediately above the foot, neither in the cells of the columella nor in the cells of the epidermis. The plastids of these cells differ also in shape, being ovoid or irregularly elongated rather than disc-shaped. In many of the chloroplasts of the cells of the region of intercalary growth large vacuoles are to be seen, while in the plastids of the spores no protoplasmic contents are to be recognized, the plastids having become, as Davis says, "storage vesicles of starch." These cells lacking pyrenoids nearly all have some starch present within them, varying from a single grain in the youngest archesporial cells to many grains in the mother cells and spores.

All of the cells of the "archesporium" contain single minute chloroplasts which are in many cases difficult to distinguish from the granular cytoplasmic cell contents (figure 9). They contain a few starch grains, but as stated above, no pyrenoid or other conspicuous bodies are to be identified. The ground substance of the chloroplast stains very slightly, appearing to have almost no granular contents. Later as these cells enlarge to form the spore mother cells, the plastids also enlarge and minute, elongated or flattened bodies (figure 10), are clearly to be seen scattered throughout the chloroplast. There is no doubt that if the "Anlagen" of these bodies were present in the

plastids in the archesporial cells they were too minute to be distinguished with the highest magnifications. So far as it is possible to determine, they have arisen *de novo*. They take the blue stain and are probably starch masses. Their shape and size is essentially the same as that of the pyrenoid bodies seen in assimilative cells. Whether they ever at any earlier period stain red as do the pyrenoid bodies I have been unable to determine. That they develop directly into the large starch grains to be seen in the spores is easily proven. Figure 11 shows a chloroplast at a somewhat later period in which these bodies have enlarged greatly and are now without question starch grains. There seems thus to be no doubt that two different modes of starch formation exist in Anthoceros, the one occurring in connection with the pyrenoid, in cells active photosynthetically, and the other occurring in cells lacking pyrenoids, in which the carbohydrate is being deposited only. I have unfortunately up to the present been unable to get satisfactory material to test this experimentally.

The chloroplasts of the cells of the assimilative tissue surrounding the sporogenous layer immediately above the foot contain numerous scattered starch grains but no traces of a central aggregation of smaller bodies (figure 12). If the bodies which later aggregate to form the pyrenoid are present in these cells though scattered, they are either unrecognizable among the starch grains or they are too minute to be detected. In some of the slightly older cells of this same layer, minute bodies, which may be rudimentary pyrenoid bodies (figure 13), are to be seen scattered through the plastid. In the central region of other chloroplasts are loose aggregates of similar bodies which suggest the pyrenoid (figure 14). Along with these bodies—usually nearer the periphery of the plastid—are to be seen scattered starch grains which seem to be the same in form and structure as those seen in tissues of the sporophyte and gametophyte which are exposed to abundant light. As will be seen from figure 15 these starch grains are much larger and are easily distinguishable from the minute centrally located pyrenoid bodies. Still higher up in the region of intercalary growth the plastids show definite central aggregations of reddish stained bodies with but few or no starch grains in the peripheral region (figure 16). Higher up the form of the chloroplasts and pyrenoids becomes the same as that of those already described for the gametophyte (figure 17). Those plastids in the regions of the sporophyte receiving full exposure to the light seem to be identical with

those of the gametophyte. And as is to be expected, the structure of the pyrenoid, the mode of starch formation from it, as well as the distribution of the starch, are also the same.

Thus it will be seen that all chloroplasts actively engaged in photosynthesis have the same structure. On the other hand those chloroplasts in which photosynthesis has not yet begun, as in the embryonic area of the sporophyte, and in those cells having other functions than photosynthesis as the sporogenous cells and those of the foot, the pyrenoid is lacking. In these latter cells starch is deposited in considerable amounts but not through the agency of a visible pyrenoid.

The protoplasm of the less active plastids, both of the sporophyte and the gametophyte, is usually of a compact texture showing very fine and uniform reticulation (figure 3). Plastids in which starch is being formed rapidly have a texture less uniform and regular. The reticulations are coarse and elongated in the direction of the long axes of the starch grains (figure 7). The presence earlier of starch grains in this protoplasm is no doubt responsible for this peculiarity in its texture.

The chloroplasts of Anthoceros multiply by fission as is usual with the plastids of other plants. Seen in cross section the plastid seems to elongate, and the pyrenoid to elongate with it (figure 18). The pyrenoid finally separates into two parts and we have a much elongated chloroplast with a pyrenoid in each end (figure 19). This plastid pinches in two and forms the new plastids. The chloroplast shown in figure 19 is an unusually large one found in the older part of the thallus.

As a type of the mode of starch formation in the Marchantia group, I will here refer briefly to this process in *Reboulia hemispherica*. I hope later to give a detailed account of starch formation in some of the members of this group.

The chloroplasts of the cells of this liverwort are spheroid or ovoid bodies having an average diameter of about 6 microns. The average number in each cell seems to be from 8 to 16.

The protoplasm of those plastids which lack starch is irregularly distributed. Scattered vacuole-like areas are present in which no granular protoplasm is to be detected (figure 20). In the chloroplasts which are partly filled with starch grains these clearer, homogeneous areas are less conspicuous or lacking (figure 21). No deeply stained bodies other than the starch bodies are present, either aggregated or scattered. The smallest stainable body to be detected in these plastids seems to be starch.

The starch is formed with no apparent increase in the size of the chloroplast. It is formed in disc-shaped masses which may lie in any part of the plastid and in any position. More commonly they lie near the periphery of the plastid or they may extend across the central region as flattened bi-convex discs (figures 21 and 22). Usually from three to ten of these disc-shaped starch grains are present. Plastids filled with starch have much the same general appearance as plastids to be found in the region of the foot in the sporophyte of Anthoceros (figure 12). Those of Reboulia are however smaller and have fewer starch grains. These figures of the plastids of Reboulia are very similar to the well known figures of Sachs for Funaria, both as to the shape of the starch grains and as to their distribution.

Discussion

As has been shown, the so-called pyrenoid of Anthoceros is in no case homogeneous as in the green algae but is at all times a more or less crowded mass of disc or spindle-like bodies. Lutman (11) has found that in Closterium the entire pyrenoid occasionally breaks up "into lens-shaped segments." In this segmented condition these pyrenoids may remotely resemble the multiple pyrenoids of Anthoceros. It seems quite possible however that these lens-shaped segments are indicative of the conversion of the entire pyrenoid into a number of starch grains as has been described for Oedogonium and Rhizoclonium (21) and for Tetraspora (12). In any case they are not persisting structures of the plastid as is the case with the pyrenoid bodies of the pyrenoid of Anthoceros.

On the other hand the chemical nature of the bodies making up the Anthoceros pyrenoid seems to be the same as that of the pyrenoids found in the algae. Both give positive results with microchemical tests for protein. These protein bodies serve as the foundations or the groundwork of the starch grains and are converted into starch with no change in form, and, at first, no change in size. According to Timberlake (21) the pyrenoids of Rhizoclonium, Oedogonium, and Cladophora become at times entirely transformed into a single large mass of starch. This is also frequently the case in Tetraspora (12). The mode of starch formation from these pyrenoids is then at times the same as it is in Anthoceros from the single pyrenoid bodies. It must not be lost sight of however that here the comparison is between the entire pyrenoid of the above mentioned algae and a single pyrenoid

body of Anthoceros. Based upon these comparisons the pyrenoid of Anthoceros would in reality be a compact group of minute pyrenoids, that is to say a "multiple pyrenoid."

The pyrenoid of Anthoceros, then, though differing strikingly in structure from those of the green algae, has nevertheless much in common with them. The published work indicates that the structures which have previously been termed "pyrenoids," though apparently all kernel-like protein bodies, are nevertheless not all alike in their relation to starch formation. While those present in the chloroplasts of the diatoms, in certain of the Rhodophyceae, and the Euglenidae may be concerned in the formation of other carbohydrates, true starch has not been demonstrated in any members of these groups. According to Schmitz (19) and Schimper (18) the Floridian starch appearing in many of these forms seems to be formed with no apparent connection with the pyrenoids or even with the plastids. In the Chlorophyceae the work of Timberlake has shown that the pyrenoid takes a direct morphological part in the process of starch formation, at least in the forms studied by him. Segments of the pyrenoid are split off to form the rudimentary starch grains (20) or the entire pyrenoid, as cited above, may form a single large starch mass (21). And while Lutman (11) was unable to arrive at a definite conclusion as to the mode of starch formation in Closterium, his work at least offers no support to the earlier conclusions of Schmitz (19) and of Schimper (18) that the pyrenoid although closely related to starch formation, does not itself take any direct, morphological part in the process. It is probable that more weight should be attached to the more recent work because of the great advances in microscopic technique in the last thirty years.

There are no visible structures in the chloroplasts of the other Bryophyta that are analogous to the pyrenoid bodies of Anthoceros. As described above their chloroplasts are considerably smaller and do not show in any case any visible products of photosynthesis previous to the appearance of the starch grains. No bodies are visible which serve as the beginnings of the starch grains, either scattered or aggregated in a mass. The smallest stainable bodies of the plastid stain the same color as the large starch grains and are therefore quite probably starch. . As in Anthoceros the size of the chloroplasts remains apparently the same whether they are free from starch or partly filled with it.

The starch in the sporogenous cells and in other cells of the sporophyte of Anthoceros which lack pyrenoids, seems however to be formed in much the same manner as in Reboulia and the other Marchantia forms. This is probably also the case with those Anthoceros species with multiple chloroplasts which lack pyrenoids, although in these plastids photosynthesis takes place while in the above mentioned plastids of the sporophyte the starch is probably deposited from soluble carbohydrate formed elsewhere.

Campbell's work (3, 4) on certain tropical Anthoceros forms would seem to indicate that some relation exists between the size of the chloroplasts and the presence of pyrenoids in them. The conditions in *Megaceros Salakensis* are especially suggestive, where the single chloroplast of the surface cells contains a poorly defined pyrenoid, while the four to six plastids of the deeper lying cells contain no pyrenoids. It is, of course, to be expected that small chloroplasts would contain correspondingly smaller pyrenoids, but their entire disappearance is unexplainable. The pyrenoid is to be seen in every cell of the gametophyte of *Anthoceros laevis* whether in the small chloroplasts in the region of the growing point or in those relatively large chloroplasts of the surface cells of active, mature thalli. Here the size of the chloroplast merely determines the size of the pyrenoid. On the other hand pyrenoids are lacking in all of the cells of the foot and the embryonic tissue of the sporophyte. Although they later become evident in the assimilative tissue they are never to be identified in the cells of the sporogenous layer. The cause of the absence of pyrenoids in the chloroplasts of the embryonic cells of the sporophyte and of the sporogenous layer may be due to a failure to form starch directly by photosynthesis in these cells.

Since Campbell's references to the pyrenoids and starch in the chloroplasts of *Megaceros Tjibodensis* and *M. Salakensis* were only incidental, his attention having been directed mainly to the grosser morphological details, it seems very much to be desired that these forms be reinvestigated to determine if possible the relation existing between the size and the number of the chloroplasts and the presence or absence of pyrenoids.

Denniston (6) has shown that in the transformation of the soluble carbohydrates to starch in the leucoplasts of Canna and Dieffenbachia and in the chloroplasts of *Pellionia Daveauana* there is a visible intermediate product which stains orange with Flemming's triple stain.

This orange stained zone he believes to be less complex chemically than the starch. He shows that this orange stain is characteristic of immature cell plates and of cell walls which are being dissolved. It is probable that at times the pyrenoid bodies of Anthoceros, as well as parts of the pyrenoid of Hydrodictyon and probably other algae, may be regarded as intermediate products of starch formation. This intermediate product reacts microchemically as protein and therefore probably has a complexity considerably greater than that of either the soluble or the insoluble carbohydrates. If it can be shown that in the case of the leucoplast the intermediate product is really a carbohydrate not greatly different from starch then it would seem that starch formation in the presence of a pyrenoid is quite a different process in which more complex reactions involving a protein stage seem to be necessary to the production of the carbohydrate. Since starch may be deposited in chloroplasts as well as in leucoplasts in the absence of light, it is quite possible that normally it is formed from soluble carbohydrate without the intervention of photosynthesis.

The phenomenon of starch formation from the pyrenoid bodies of Anthoceros seems to support the view held by Schimper (18) and Eberdt (7) that starch is formed by a transformation of protoplasm. Certainly starch which is formed by the direct transformation of a protein body can hardly be said to be secreted from the protoplasm, as is held to be the case by Meyer (13) and by Salter (15).

The formation of new pyrenoids in Anthoceros is normally by a separation of a preexisting pyrenoid into two halves during the fission of the chloroplast. New pyrenoid bodies are apparently formed similarly, that is, by the division of other pyrenoid bodies. In the embryonic tissue of the sporophyte however we have what clearly seems to be a formation of pyrenoid bodies and pyrenoids *de novo*. Formation of pyrenoids *de novo* is not uncommon in the green algae and probably should not be unexpected here. The formation of pyrenoids here is however by the aggregation of scattered pyrenoid bodies which have themselves apparently arisen *de novo*. The origin of the pyrenoid bodies from scattered parts of the chloroplast is suggestive and may indicate that their "Anlagen" have been present from the beginning of the sporophyte but scattered and not readily stainable. Such a hypothesis as this might be used to explain the formation of starch in the sporogenous cells where no visible pyrenoid is present.

SUMMARY

1. The pyrenoid of *Anthoceros laevis* is not homogeneous at any time but is made up of from 25 to 300 closely aggregated disc- or spindle-shaped bodies, which I have called pyrenoid bodies. These pyrenoid bodies give positive results with microchemical tests for protein.

2. During photosynthesis the outer bodies of the pyrenoid are converted directly into starch masses. These rudimentary starch grains increase in size to form the mature starch grains.

3. New pyrenoid bodies are formed apparently by the fission of preexisting bodies.

4. In the sporogenous tissue of the sporophyte, starch is formed in large amounts without the agency of visible pyrenoids.

5. Pyrenoids are not visible in the embryonic assimilative tissue of the sporophyte, but, as these cells are pushed away from the embryonic region, scattered bodies appear which later become closely aggregated to form the pyrenoid.

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EXPLANATION OF FIGURES IN PLATE VIII

All figures were drawn with the aid of a camera lucida. A Zeiss 2 mm apochromatic objective with an 8 compensating ocular (magnification about 1,400) was used for all figures except Figs. 7, 9, 20, 21, and 22 which were drawn with a 12 ocular.

FIG. 1. A somewhat flattened chloroplast situated in a deeply lying cell of a mature thallus. The pyrenoid is compact and is made up of bodies which stain a dull red.

FIG. 2. A large, thick chloroplast from a surface cell of a mature, active thallus. The pyrenoid is cut slightly tangentially so that not all of the bodies appear as discs.

FIG. 3. A very thin chloroplast from a surface cell of a thin thallus.

FIG. 4. A surface view of a large, active plastid. The faces of the starch grains show rather than the edges and the pyrenoid bodies appear less angular.

FIG. 5. A large plastid cut slightly obliquely.

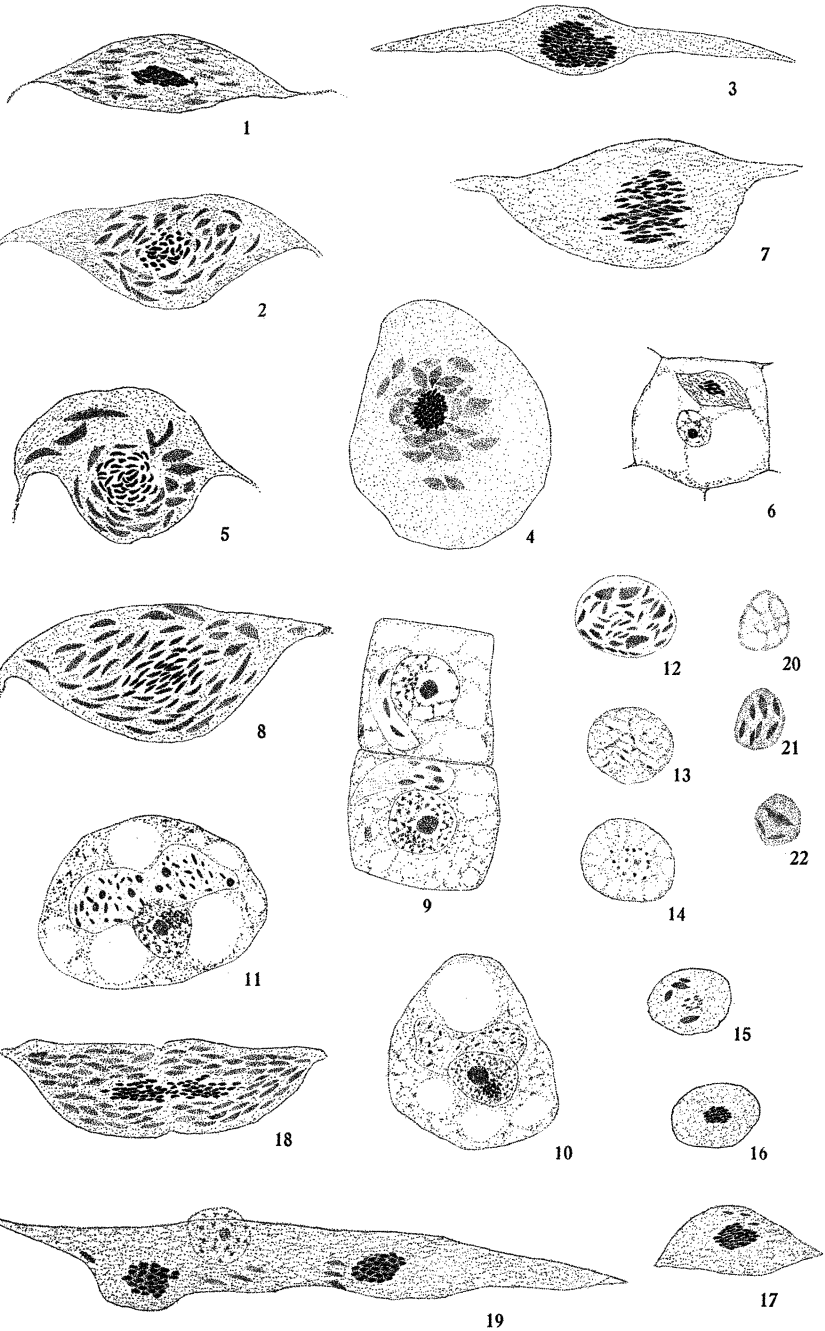
FIG. 6. A cell from near the growing point of the thallus showing the small chloroplast with its pyrenoid made up of not more than 25 to 30 pyrenoid bodies.

FIG. 7. A plastid in the vicinity of the growing point showing the pyrenoid bodies apparently multiplying by fission.

FIG. 8. A plastid in which there is a gradual transition from the loose mass of brilliantly red-stained pyrenoid bodies into the blue stained starch grains.

FIG. 9. Two archesporial cells just above the foot which show the single indistinct plastids.

FIG. 10. A spore mother cell the single plastid of which has many scattered bodies stained deeply with the violet stain. These bodies are probably very minute starch grains.



McALLISTER: PYRENOID OF ANTHOCEROS.

FIG. 11. A spore mother cell somewhat older, with the plastid filled with many small starch grains which seem to have developed from the minute violet-stained bodies of Fig. 10.

FIG. 12. A plastid in the embryonic area of the sporophyte immediately above the foot, outside the sporogenous layer. No pyrenoid is visible though the plastid is filled with starch grains showing great variation in size.

FIG. 13. A slightly older plastid from the same region as Fig. 12 which has no starch but has scattered stained bodies which may be rudimentary pyrenoid bodies.

FIG. 14. A chloroplast older than that in Fig. 13 in which a number of minute bodies are to be seen in the central region,—probably scattered pyrenoid bodies.

FIG. 15. A slightly older stage in which the pyrenoid can be identified with certainty.

FIG. 16. A plastid in the upper limits of intercalary growth which has a conspicuous dull red pyrenoid.

FIG. 17. A chloroplast somewhat older than that shown in Fig. 16, at the upper edge of the gametophytic sheath.

FIG. 18. A large plastid in a gametophytic cell, beginning to divide.

FIG. 19. A very large plastid of an interior cell of the thallus, with the pyrenoid divided but the plastid still undivided.

FIG. 20. Chloroplast of Reboulia hemispherica lacking starch.

FIGS. 21 AND 22. Chloroplasts of Reboulia showing the common form and distribution of the starch grains.